

# A Pharmacokinetic Model of Inhaled Methanol in Humans and Comparison to Methanol Disposition in Mice and Rats

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We estimated kinetic parameters associated with methanol disposition in humans from data reported in the literature. Michaelis-Menten elimination parameters ( $V_{\max} = 115$  mg/L/hr;  $K_m = 460$  mg/L) were selected for input into a semi-physiologic pharmacokinetic model. We used reported literature values for blood or urine methanol concentrations in humans and nonhuman primates after methanol inhalation as input to an inhalation disposition model that evaluated the absorption of methanol, expressed as the fraction of inhaled methanol concentration that was absorbed ( $\Phi$ ). Values of  $\Phi$  for nonexercising subjects typically varied between 0.64 and 0.75; 0.80 was observed to be a reasonable upper boundary for fractional absorption. Absorption efficiency in exercising subjects was lower than that in resting individuals. Incorporation of the kinetic parameters and  $\Phi$  into a pharmacokinetic model of human exposure to methanol, compared to a similar analysis in rodents, indicated that following an 8-hr exposure to 5000 ppm of methanol vapor, blood methanol concentrations in the mouse would be 13- to 18-fold higher than in humans exposed to the same methanol vapor concentration; blood methanol concentrations in the rat under similar conditions would be 5-fold higher than in humans. These results demonstrate the importance in the risk assessment for methanol of basing extrapolations from rodents to humans on actual blood concentrations rather than on methanol vapor exposure concentrations. **Key words:** human-animal comparison, methanol, physiologically based pharmacokinetic model, toxicokinetics. *Environ Health Perspect* 103: 726-733 (1995)

Methanol is a widely used industrial solvent. Workplace exposures to high methanol vapor concentrations have resulted in acute toxicity, including central nervous system depression and blindness; large oral doses of methanol have resulted in blindness and death (1). Investigations of methanol inhalation in laboratory rodents have indicated that high doses of methanol cause a variety of birth defects (2-7). As methanol is proposed as a motor fuel additive, the general public may be exposed to increased levels of environmental methanol, prompting recent attempts to assess the risk of the teratogenic potential of methanol in humans (8). A logical first step in the assessment of risk to the human conceptus, as compared to the rodent conceptus, is to evaluate the dose of

methanol actually administered to the mother via inhalation and the time course of exposure in maternal blood. The dose administered is not the same as the inhaled concentration because the inhaled toxicant may not be absorbed completely. If there are differences in the absorption of gaseous or vapor-phase toxicants between species, these differences should be considered in risk assessment. Analyses of the absorption of water-soluble vapors such as methanol have suggested that the amount absorbed varies, and that the benchmark physiologically-based pharmacokinetic model developed for inhaled styrene does not provide accurate predictions of the absorption of highly water-soluble substrates (9).

In earlier work in this laboratory (10; Perkins et al., submitted), the systemic disposition of methanol in female Sprague-Dawley rats and CD-1 mice was evaluated, the ventilation in rodents was quantified in flow-through exposure chambers, and uptake of methanol vapor from the chamber atmosphere was determined. These data allowed development of an inhalation pharmacokinetic model based on the equation:

$$\frac{dC}{dt} = \frac{\Phi \dot{V} C_{\text{inh}}}{V_d} - \frac{V_{\max}}{V_d} \left( \frac{C}{K_m + C} \right), \quad (1)$$

where  $C$  is the blood methanol concentration,  $\Phi$  is the fraction of inhaled methanol absorbed into the systemic circulation,  $C_{\text{inh}}$  is the ambient methanol vapor concentration (assuming a well-mixed environment),  $\dot{V}$  is the minute ventilation,  $V_d$  is the volume of distribution, and  $V_{\max}$  and  $K_m$  are the Michaelis-Menten parameters associated with the maximum velocity of metabolism and the concentration at which velocity is one-half maximum, respectively. In both the rat and mouse, methanol elimination occurred via apparent Michaelis-Menten kinetics, with a similar  $K_m$  (approximately 50 mg/L) in both rodent species;  $V_{\max}$  was approximately twice as large in the mouse as in the rat (154 mg/kg/hr versus 71 mg/kg/hr) (10). The absorption factor  $\Phi$  was not constant in the rat, but varied with both  $\dot{V}$  and  $C_{\text{inh}}$  (Perkins et al., submitted). Elimination via the lungs (after exposure) and the kidney is theoretically a small fraction of total

methanol elimination (8,11) and modeled well in both rat and mouse without being specifically included (10). In both the rat and mouse, the systemic disposition of methanol was similar after oral (PO), intravenous (IV), or inhalation administration (Perkins et al., submitted).

To assess risk to the human conceptus based on extrapolation from rodent data, it is desirable to begin with comparisons of blood methanol concentrations rather than with the ambient methanol vapor concentration; as demonstrated in rodents, blood concentrations can vary substantially between species exposed to the same ambient methanol concentration. Blood toxicant levels are a more relevant determinant of systemic effects (such as teratogenicity) than are ambient environmental toxicant concentrations (12). The objective of this study was to apply the inhalation toxicokinetic model developed for methanol exposure in rodents to humans, based on blood methanol data extracted from the literature. This effort relied on evaluation of published kinetic parameters after IV or PO methanol administration to humans and other primates, estimation of  $\Phi$  from published methanol inhalation studies and physiological parameters, application of the model to the data, determination of which model parameters (if any) required adjustment to optimize description of the concentration-time data, and comparison of blood methanol concentrations predicted by the human model to observed data in rodents. In addition, predictions based on the human model were evaluated for implications to risk assessment.

## Materials and Methods

Because of the well-established species differences in the acute toxicity of methanol between primates and nonprimates (1), the primate is the model of choice for both kinetic and toxicologic studies of methanol, and only data obtained from human and nonhuman primates in the published literature were used in this study. Numerical values presented in tables were preferred to values that were scaled from charts or graphs, although the majority of data were scaled. Many of the data were reported as methanol concentrations in urine rather than in blood. It has been shown that blood methanol concen-

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trations are 77% of urine methanol concentrations (13); similar values were reported by other investigators (8,14) and correspond to earlier work in this laboratory (10). Urine data reported by other investigators (15–17) were corrected for specific gravity and/or blood creatinine, although little advantage was noted in these corrections. We therefore used uncorrected urine data.

The general procedure used was to evaluate systemic kinetic parameters based on data after PO or IV methanol administration, then to fit the inhalation data sets with Equation 1, maintaining  $\Phi$  as the dependent variable. Data after IV administration were modeled with a one-compartment system incorporating saturable elimination as

$$\frac{dC}{dt} = -V_{\max} \left( \frac{C}{K_m + C} \right); C_0 = \frac{X_0}{V_d}, \quad (2)$$

where  $C$  is the methanol concentration in the central compartment (18). The initial condition ( $C_0$ ) is the mass of methanol administered ( $X_0$ ) divided by the apparent volume of distribution ( $V_d$ ). For PO administration, the model described above was modified to take into account absorption of methanol from the gut. The rate of change of blood methanol concentration after PO administration is

$$\frac{dC}{dt} = \left[ \frac{X}{V_d} (K_a) \right] - \left[ V_{\max} \left( \frac{C}{K_m + C} \right) \right], \quad (3)$$

where  $K_a$  is the first-order rate constant for absorption from the gastrointestinal tract. The elimination terms are the same as Equation 2 above.

At low values of blood methanol, the saturable elimination in Equation 2 becomes approximately first order:

$$\frac{dC}{dt} = -K_{el}(C), \quad (4)$$

where the first-order rate constant  $K_{el}$  is equal to the ratio of  $V_{\max}$  to  $K_m$ . Use of both saturable and linear models facilitates data acquisition from the literature because blood concentrations after low-dose methanol administration frequently are characterized by the first-order elimination rate constant or the associated half-life.

One of the data sets from the literature that described high methanol inhalation exposures of industrial workers displayed some anomalously high blood methanol concentrations, which implied that the subjects were eliminating methanol at a much lower rate than expected. The ethanol co-administration model was developed to

determine if relevant PO doses of ethanol, co-administered with the methanol inhalation exposure, could account for the anomalies. The ethanol co-administration model assumed direct administration into the central compartment, similar to IV dosing, for simplicity. The model is identical to Equation 1 but with two elimination terms, one for methanol and one for ethanol. The kinetic parameters used in the model for ethanol were  $V_{\max} = 230$  mg/L/hr,  $K_m = 82$  mg/L, and  $V_d = 0.54$  L/kg (19). For methanol elimination, the kinetic parameters are as discussed below, except that  $V_{\max}$  was modeled as a function of the ratio of ethanol to methanol in the blood. Based on earlier work which reported 90% inhibition of alcohol dehydrogenase (ADH)-mediated methanol metabolism when ethanol and methanol are at equimolar concentrations and 70% inhibition when ethanol concentrations are 25% of methanol concentrations (20), a simple model was developed that predicted 90% inhibition of methanol metabolism at ethanol concentrations greater than methanol concentrations, a linear variation from 70% to 90% inhibition as the molar ratio increased from 0.25 to 1.0; and a linear increase in inhibition from 0.0% to 70% as the molar ratio increased from 0 to 0.25.

Except as noted, the  $\dot{V}$  used was for a body weight of 70 kg, as reported for human volunteers in a chamber equipped with a two-way non-rebreathing valve (21) or a respirometer (22). This  $\dot{V}$  is higher than the estimate of 6–7.5 L/min reported in standard texts (23), but was chosen as being more representative of nonexercising human volunteers in chambers than humans at rest. Typical  $\dot{V}$  of  $10.3 \pm 4.2$  L/min were reported for nine human subjects in average classroom and workshop settings, measured with noninvasive instruments (i.e., no mouthpiece or forced breathing) (24). The  $\dot{V}$  of 10.5 L/min, normalized for published body weight, also was used for the nonhuman primates.

The simulations were performed on a spreadsheet program by converting the differentials to finite differences with a time step of 0.1 hr. Where continuous, instantaneous values were required (e.g., the blood concentrations in the Michaelis-Menten equation), the blood concentration from the previous time step was used. Background blood methanol in the human is approximately 1.0 mg/L from both endogenous and exogenous sources (8), and this level was used for the initial time step. Studies based on 0.01-hr time steps indicated no significant loss of accuracy by use of the larger time step. Nonlinear least-squares regression, where required, was performed with PCNONLIN (Statistical Consultants, Inc., Lexington, Kentucky).

## Results

Several investigators have reported a  $K_{el}$  for methanol of approximately  $0.25 \text{ hr}^{-1}$  (25) or  $0.23\text{--}0.28 \text{ hr}^{-1}$  (1) in humans. Other investigators, however, reported a  $K_{el}$  of  $0.101 \text{ hr}^{-1}$  for oral doses of 3, 5, or 7 mL of methanol (13). Those authors assumed that 100% of the dose was absorbed within 1 hr, and they did not otherwise account for absorption of methanol from the gut. Their methanol blood concentration–time data were fit with the one-compartment model with first-order absorption (Equation 3) by nonlinear regression, which yielded a  $K_{el} = 0.25 \text{ hr}^{-1}$ , a  $K_a = 2.15 \text{ hr}^{-1}$ , and a  $V_d = 0.84$  L/kg. The estimates of both  $K_a$  and  $V_d$  were similar to corresponding constants in the mouse and rat [ $K_a = 2.11$  for rats, and 2.98 for mice (average of fast and slow absorption processes);  $V_d = 0.85$  for rats and 0.76 for mice] determined previously in this laboratory (10).

In previous investigations, a Lineweaver-Burke plot based on methanol doses in the rhesus monkey  $0.05\text{--}1.0$  g/kg (producing blood methanol concentrations of approximately 60 to 1200 mg/L) was used to recover an apparent  $K_m$  of 278 mg/L, based on  $V_d$  of 0.7 L/kg (20). In other work, a  $K_m$  of 544 mg/L for methanol was reported with the monkey ADH enzyme *in vitro* (26). Still other investigators estimated the  $K_m$  for ethanol in humans and assumed, based on the much weaker binding of methanol to ADH, that the  $K_m$  for methanol should be 640 mg/L (27). Based on the average of these estimates (460 mg/L) and a  $K_{el}$  of  $0.25 \text{ hr}^{-1}$ ,  $V_{\max}$  was estimated for this investigation to be 115 mg/L/hr.

Nonlinear regression analysis of available data is presented in Table 1. The range of  $K_m$  estimates was between 252 and 716 mg/L, with a mean of 484 mg/L (similar to the average value of 460 mg/L noted above). Since the monkey is a good model for human methanol metabolism,  $K_m$ , which is a function of enzyme binding, should be approximately the same between primate species and genetically and environmentally diverse humans.  $V_{\max}$  is expected to vary at least somewhat between individuals and species due to differences in the total amount of enzyme. A  $K_m$  of 460 mg/L was selected and used in Equation 3 to calculate the concomitant  $V_{\max}$  of 115 mg/L/hr, which was incorporated into all modeling except as noted. Figure 1 shows the fit of Equation 2, incorporating those values of  $V_{\max}$  and  $K_m$  to data sets obtained from the literature (28–30). The reasonable correspondence between model estimates and observed data suggested that the parameter values estimated above were suitable for further evaluations.

The model (Equation 1) was used to determine the value of  $\Phi$  that provided the

best fit of the available data. That is,  $\Phi$  was evaluated as the dependent variable using the exposure concentration as the independent variable and  $V_d$ ,  $V$ ,  $V_{max}$ , and  $K_m$  as known parameters for each value of  $C_{inh}$  in the data sets. Data obtained during inhalation exposures were divided into three groups: low dose (50–300 ppm), mid-dose (500–2000 ppm), and high dose (>2000 ppm) for modeling purposes.

**Low dose.** Groups of three or four healthy humans were exposed for 8 hr to environments of 77, 156.5, and 229 ppm methanol in air, and urine methanol concentrations were determined (22). Figure 2 shows the estimated blood methanol concentrations in those subjects and model predictions with the parameters defined above and  $\Phi = 0.645$ ; that value of  $\Phi$  yielded exactly the 8-hr blood methanol concentration of the 156.5 ppm dose. Those authors also reported a “retention factor,” which is equivalent to a  $\Phi$  of 57.7%. This factor was deduced by having the subjects, who had their noses clipped shut, breathe through a two-way valve; the difference between inhaled and exhaled methanol concentrations was then measured.

Other investigators (21) exposed four subjects to 200 ppm methanol, the current allowable exposure threshold limit value (TLV) for workers (31). Two different experiments measured subjects either at rest or during exercise. Ventilation was measured with a two-way non-rebreathing valve. These data are reproduced in Table 2, along with the  $\Phi$  that was required to fit the methanol inhalation model to these data. Although the difference in blood methanol concentrations at 6 hr between the exercise and resting groups was not statistically significant, the computed  $\Phi$  indicated that fractional methanol absorption was much lower in the exercise group.

**Mid-dose.** Individual subjects were exposed to approximately 500 and 1000 ppm methanol for relatively short durations (approximately 2–5 hr) (13). The observed blood methanol concentrations are compared to model predictions in Figure 3. A different value of  $\Phi$  was required to fit the data for each subject. The data from one subject, who weighed 57 kg, required  $\Phi = 0.79$ ; the other subject, who weighed 78.5 kg, required  $\Phi = 0.67$ – $0.70$ .

Other investigators (32) exposed groups of 4 male rhesus monkeys to 50, 200, 1200, or 2000 ppm methanol for 6 hr and reported the resulting blood concentration at the end of exposure and for the subsequent 18 hr. No increase in blood methanol concentration for the 50 ppm group was reported, and concentrations in the 200 ppm group were close to background and therefore difficult to interpret. For the 1200 and 2000 ppm exposures, the

methanol inhalation model estimated a  $\Phi$  of 0.69 (data not shown).

**High dose.** Several articles (15–17) presented the results of examinations of workers in Japan, some of whom were exposed

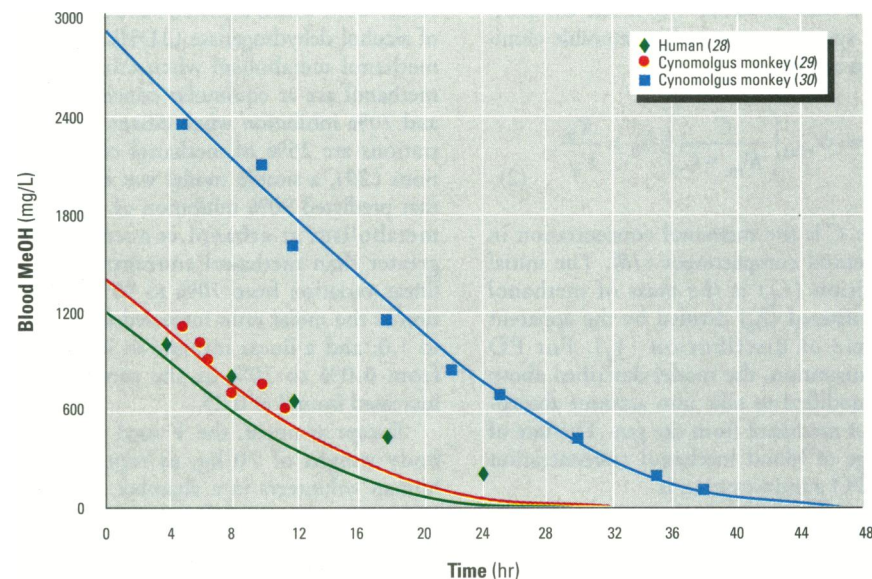
to high methanol vapor concentrations (up to 5000 ppm), yielding very high blood methanol concentrations (>250 mg/L). Those investigators provided linear regression analysis of blood or urine methanol

**Table 1.** Nonlinear least-squares regression analysis of four methanol oral or intraperitoneal exposures (means  $\pm$  SE)

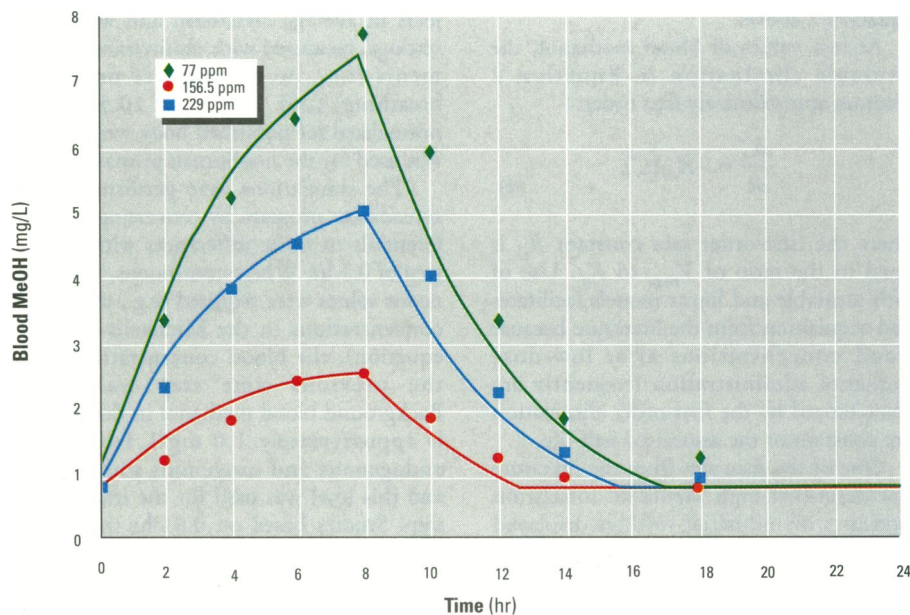
$V_{max}$	$K_m$	Dose, species	Reference
132 $\pm$ 36	716 $\pm$ 489	2 g/kg, cynomolgus monkey	(29)
93 $\pm$ 87	320 $\pm$ 1273	1400 mg/L, human <sup>a</sup>	(28)
48	278	Various doses, rhesus monkey <sup>b</sup>	(20)
59 $\pm$ 7	252 $\pm$ 116	1 g/kg, cynomolgus monkey	(30)

<sup>a</sup>Blood methanol concentration shortly after admission to hospital.

<sup>b</sup>Doses of 0.05–1.0 mg/kg given;  $V_{max}$  and  $K_m$  reported, based on analysis of Lineweaver-Burke plot.



**Figure 1.** Demonstration of one-compartment model with Michaelis-Menten elimination (Eq. 2), with  $V_{max} = 115$  mg/kg/hr and  $K_m = 460$  mg/L (lines) to the data (symbols) for humans (28) and cynomolgus monkeys (29,30).  $C_0$  calculated by dose/ $V_d$  if dose was given, or calculated by regression.

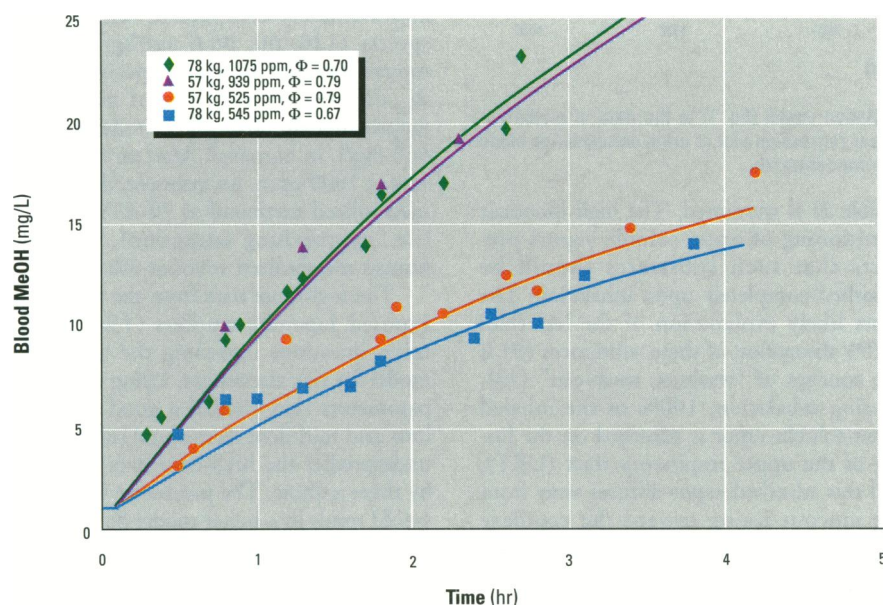


**Figure 2.** Demonstration of methanol inhalation model (Eq. 1) using  $\Phi = 0.645$  (lines) to the low-exposure data (symbols) (22).



**Table 2.** Summary of absorption factor,  $\Phi$ , deduced from reported human data and measured in rodent experiments

$C_{inh}$ (ppm)	$\Phi$	Notes	Reference
Low exposure			
77–229	0.645	$\dot{V} = 10.5$ L/min, measured	(21)
200	0.740	$\dot{V} = 10.5$ L/min, measured	(22)
200 (exercise)	0.50	$\dot{V} = 18.6$ L/min, measured	
Medium exposure			
500–1000	0.79	57 kg subject	(13)
	0.67–0.70	78.5 kg subject	
Mouse			
2500	$0.86 \pm 0.19$		(Perkins et al., submitted)
10,000	$0.83 \pm 0.12$		
Rat			
2500	$0.88 \pm 0.16$		(Perkins et al., submitted)
10,000	$0.70 \pm 0.07$		

**Figure 3.** Demonstration of methanol inhalation model (Eq. 1) to the mid-exposure data (13). Data from similar experiments were combined (i.e., a 500 ppm and 559 ppm experiment were combined into their approximate average of 525 ppm for the plot). Lines are the model predictions, symbols are the actual data points.

concentration at the end of an 8-hr shift versus the exposure concentration (Fig. 4). The blood methanol concentrations predicted by the inhalation model were much lower than the observed data; at a  $\Phi$  of 1.0, the model underpredicted the data by 25%. Since the authors described the workplace where the samples were taken and gave no indication of heavy exercise or labor that would increase  $\dot{V}$  significantly, the only variables in Equation 1 that are likely to account for the underprediction are in the elimination term. Eight workers studied demonstrated a definite anomaly in their blood methanol concentration–time profile. First, the workers' blood methanol at the beginning of a shift (BOS) averaged 22 mg/L, compared with the 1 mg/L average in nonexposed humans (an upper 95% confidence limit on 72 unexposed controls was 3.63 mg/L). Second, after a methanol

exposure of 1288 ppm methanol (geometric mean) for 8 hr, the average end-of-shift (EOS) blood methanol concentration for these workers was 144 mg/L. Employing the kinetic parameters for elimination described above, blood methanol should have returned to background (1 mg/L) within 10.7 hr.

These data were reanalyzed from two perspectives for modeling purposes. First, because the data were gathered late in the work week, it was assumed that the EOS blood concentrations shown were the same as the EOS concentrations the day before (i.e., steady-state conditions prevailed); the  $V_{max}$  that would be required to yield the BOS blood methanol concentrations observed on the following day were calculated. The  $V_{max}$  calculated in this manner for each of the eight workers averaged  $63 \pm 13.8$  mg/L/hr. The second approach was to

**Table 3.** Blood methanol concentrations after an 8-hr exposure to methanol vapor<sup>a</sup>

Methanol (ppm)	Blood methanol concentrations (mg/L)		
	Mouse <sup>b</sup>	Rat <sup>c</sup>	Human <sup>d</sup>
100	4–5	5.5	4
200	9–12	11	7.5 <sup>e</sup>
305	15–20	17	11.5
500	29–45	31	19 <sup>f</sup>
1000	132–268	93.5	38.5 <sup>f</sup>
3078	1585–2315	558	129
5000	2976–4188	1018	224 <sup>g</sup>

<sup>a</sup>The 1000 and 5000 ppm rat and mouse data are computed for environmental exposures, but closely correspond to data from actual chamber exposures (Perkins et al., manuscripts submitted). All other data represent computed values.

<sup>b</sup>Range indicates two different ventilation patterns observed; low number represents slow breathers, high number represents fast breathers (Perkins et al., submitted).

<sup>c</sup> $\dot{V}$  as a function of blood methanol and  $\Phi$  as a function of  $C_{inh}$  is accounted for in the rat model (Perkins et al., submitted).

<sup>d</sup>Based on  $\Phi = 0.75$ .

<sup>e</sup>Approximately actual (Fig. 2).

<sup>f</sup>Approximately actual up to 4 hr (Fig. 3).

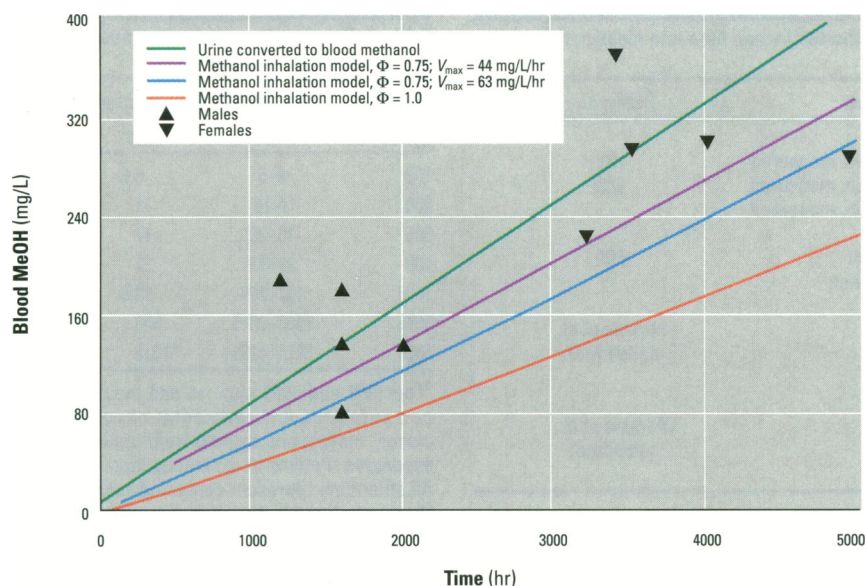
<sup>g</sup>Field work indicates 411 mg/L for 8-hr exposure to this methanol vapor concentration (see text regarding high exposures).

use the standard inhalation model (with normal ventilation and  $\Phi = 0.75$ ), but starting at time 0 with the actual average worker BOS blood methanol of 22 mg/L. The  $V_{max}$  required to yield the EOS blood concentrations then was calculated. This analysis yielded a  $V_{max}$  of 44 mg/L/hr for the average worker.

## Discussion

The kinetic modeling at low- and mid-doses of methanol vapor in humans supports earlier findings in this laboratory in the rat and mouse: there is little, if any, difference in methanol disposition during and after methanol inhalation as compared to that after PO or IV administration. The model accurately predicted the decline in methanol blood concentration following removal of the monkey (32) or the human (22) from the methanol exposure environment. Because the model used  $V_{max}$  and  $K_m$  estimates based on PO and IV administration, the kinetic constants must have been similar after inhalation.

The one-compartment methanol inhalation model with Michaelis-Menten elimination accurately predicted blood methanol concentrations after low and moderate doses of methanol vapor up to 1000 ppm [i.e., fivefold higher than the current TLV and also about fivefold higher than the maximum environmental exposure expected from the use of methanol in automobile fuel (8)]. The absorption factor ( $\Phi$ ) for nonexercising subjects varied



**Figure 4.** Comparison of the high-exposure methanol inhalation model (Eq. 1) to the data of methanol-exposed Japanese workers (15–17). The green line is a linear regression plot of urine converted to blood methanol by the factor of 0.77 (urine concentration) = blood concentration.

between 0.65 and 0.75, except for one subject, a 57-kg adult male, where a  $\Phi$  of 0.79 best described the data. A  $\Phi$  of 0.80 may be an appropriate upper bound for fractional methanol absorption in humans, with a  $\Phi$  of 0.65–0.75 representing the usual range; the model based on a  $\Phi$  of 0.75 accurately predicted all the low- and medium-dose data. Previous work demonstrated that, for exercising subjects with a ventilation 77% above resting,  $\Phi$  decreased to about 0.50 (21). This decrease in  $\Phi$  with increasing ventilation is supported by measurements with a two-way valve (22), a device that forces 100% mouth breathing and presumably increases ventilation somewhat; the resulting  $\Phi$  was 0.57, which was less than the  $\Phi$  calculated for nonexercising subjects in that study.

Earlier work in this laboratory with rats suggested a decrease in  $\Phi$  with increased ventilation (Perkins et al., submitted), but this effect was not well reproduced in rats with ventilation artificially increased by carbon dioxide loading (Perkins et al., submitted). Rats demonstrated a significant decrease in  $\Phi$  with increasing inhalation concentrations up to 20,000 ppm; this decrease was not apparent in humans at the low and medium vapor concentrations (up to 2000 ppm; Table 2). This lack of effect of exposure concentration on  $\Phi$  in humans may be a consequence of the relatively low exposure concentrations. Alternatively, methanol in the humans may behave more like it does in the mice than in rats;  $\Phi$  in mice also was independent of exposure concentration (Perkins et al., submitted).

Some discussion of the decrease in  $\Phi$  between species with increasing body size

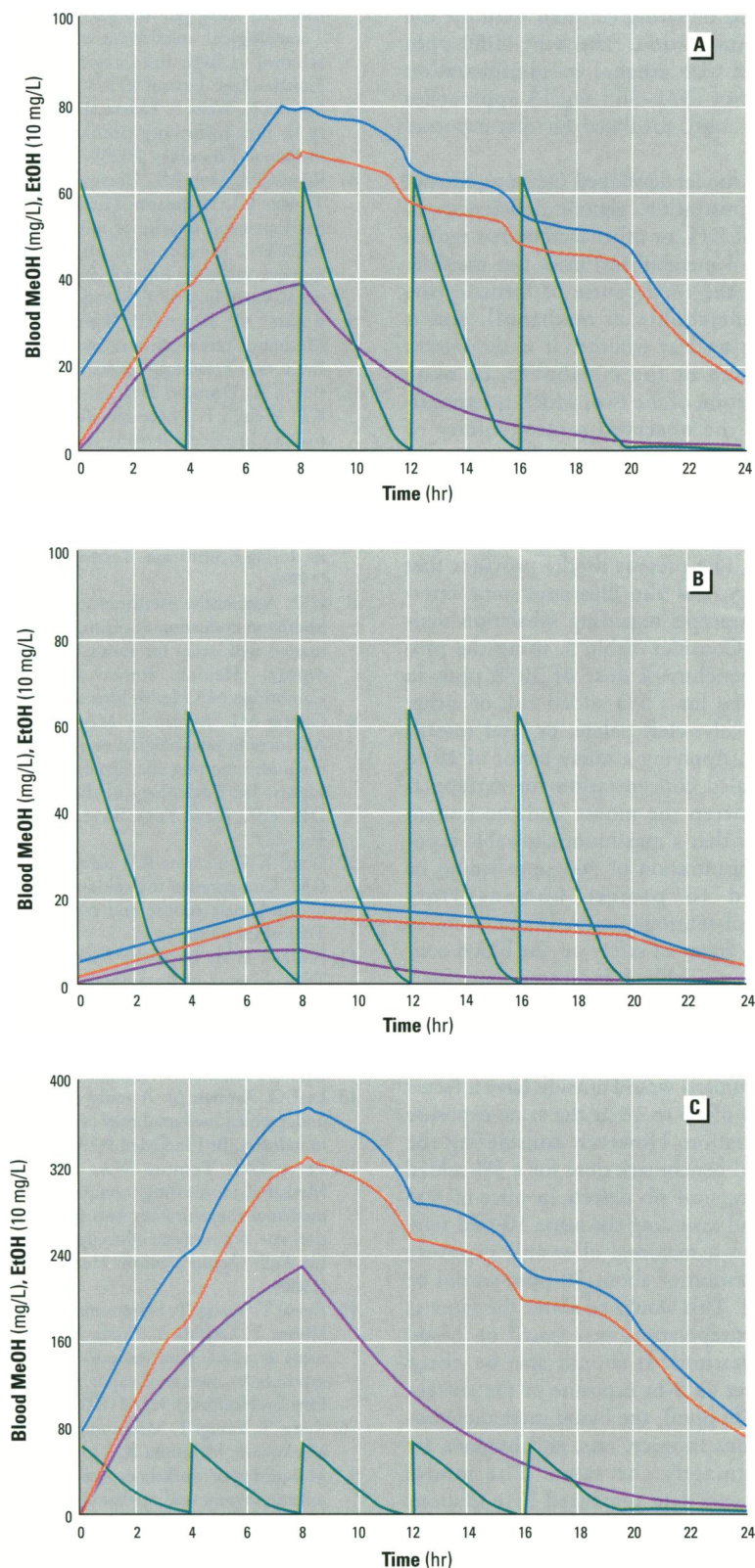
(Table 2) is warranted. The high blood:air partitioning of water-soluble vapors predicts that such substrates should be absorbed completely upon inhalation. The most likely explanation of the less than 100% absorption of these substances (9) is the concept of “wash-in, wash-out” (33). During inhalation, 100% of the inhaled water-soluble vapor is adsorbed on the lining of the upper respiratory tract (URT), and this adsorbed vapor diffuses away from the mucous lining toward the capillary blood. The diffusion process is relatively rapid but not instantaneous, so that the mucus lining still contains residual substrate when the exhaled air, devoid of substrate at the start of exhalation, re-entrains some residual substrate from the URT lining. The decrease in  $\Phi$  with increasing species size is paradoxical because the larger species breathe at a lower frequency (120, 70, and 15 breaths/min for the mouse, rat, and human, respectively). The lower breathing frequency would allow more time for the diffusion of the vapor away from the URT lining before start of exhalation, hence the fractional absorption should be higher in a slower-breathing species. One explanation for this apparent anomaly is that, normalized for body weight, the smaller species’ URT must clean, warm, and humidify larger volumes of air (1.51, 0.52, and 0.15 L/min/kg in the mouse, rat, and human, respectively) in a shorter linear distance between nares and lungs. The biological requirements for more rapid heat and water vapor mass transfer in the smaller species may be a more important factor favoring overall mass transfer than the increased time for diffusion in the slower breathing, larger species.

Table 3 illustrates two factors that are, in general, important for extrapolations used in methanol risk assessment. First, the increased ventilation per unit body weight associated with the smaller species leads to increasingly larger differences in the blood concentrations as inhaled vapor concentration increases. Ventilation per kilogram is 10-fold and 3.5-fold larger in mice and rats, respectively, than in humans; this difference is magnified because  $\Phi$  in humans is lower than in rodents. The differences in blood methanol concentrations between species, however, are not that large, especially at lower exposure concentrations (<1000 ppm), highlighting the importance of an understanding of systemic kinetics: while the  $V_{\max}$  for methanol elimination is approximately comparable for the three species (120, 60, 96.6 mg/kg/hr in the mouse, rat, and human respectively), the  $K_m$  differed by an order of magnitude (approximately 50 mg/L in rodents versus 460 mg/L in humans). After an 8-hr exposure at 1000 ppm, for example, the rodents metabolized methanol at 70–87% of  $V_{\max}$  (i.e., approaching saturation), while the human metabolized at about 8% of  $V_{\max}$ .

The high-dose data from the methanol-exposed Japanese workers (15–17) also raises questions regarding the use of the model for risk assessment. Using the model parameters that yielded a good fit of the low- and mid-dose data would substantially underpredict the high-dose data presented by these authors. The parameter values that would result in a better model fit were considered. The underprediction likely was not due to an underestimate of  $\dot{V}$  in the model: because the subjects were not exposed to heavy exercise, the value of  $\dot{V}$  used in the model was approximately 25% higher than the “at rest” estimates published in physiology texts (23) and slightly higher than that  $\dot{V}$  reported for a mixture of light activities (24). Moreover, an underprediction of  $\Phi$  is unlikely to be responsible for the underprediction of blood methanol concentrations. In rodents, an increase in inhaled methanol concentration resulted in a decrease in  $\Phi$  (Perkins et al., submitted). The value of  $\Phi$  used in the model (0.75) was higher than the mid-range of values calculated for humans (Table 2). In addition, a theoretical maximum  $\Phi$  of 1.0 still underpredicted concentrations by about 25%. While the kinetic parameter  $V_d$  could decrease with decreased cardiac output due to central nervous system (CNS) depression, clinical examination of the workers did not reveal CNS depression, nor would a decrease in volume likely have a large effect on methanol concentrations because the alcohol diffuses rapidly throughout the body water.

Variations in the elimination parameters provide the most likely explanation for





**Figure 5.** Methanol-ethanol co-administration model. (Violet lines) Predicted blood methanol concentrations during and after methanol exposure over an 8-hr period without ethanol administration; (green lines) predicted blood ethanol concentrations (1/10 scale) after 30-mL doses of pure ethanol at 0, 4, 8, 12, and 16 hr; (orange lines) predicted blood methanol concentrations during and after methanol exposure assuming beginning-of-shift (BOS) methanol levels without any residual blood concentrations (typical "Monday" exposures), after 30-mL doses of pure ethanol at the indicated times; (blue lines) predicted blood methanol concentrations during and after methanol exposure assuming BOS blood methanol concentrations accumulated over 4 consecutive days (typical "Friday" exposures) after 30-mL doses of pure ethanol at the indicated times. (A) 1000 ppm methanol; (B) 200 ppm; (C) 5000 ppm.

these data.  $K_m$  is a function of the ADH enzyme system, which is assumed to be similar in all primates.  $V_{max}$ , therefore, is the parameter most likely to be the cause of the relatively slow elimination observed in this study. In order for the model (Equation 1) to be accurate,  $V_{max}$  must be reduced to less than half the value used at lower exposures. The  $V_{max}$  and  $K_m$  (115 mg/kg/hr, and 460 mg/L) used in the modeling were chosen based on literature values of  $K_{cl}$  and  $t_{1/2}$ , which are frequently reported and can be measured accurately, and an average value of  $K_m$  reported for primates. Table 1 shows  $V_{max}$  and  $K_m$  calculated by nonlinear regression directly for primate data sets, without regard to the relationship between  $K_{cl}$ ,  $V_{max}$ , and  $K_m$  (Eq. 4). Some of the  $V_{max}$  values in Table 1 are much lower than the  $V_{max}$  used in the model, but these were associated with a correspondingly low  $K_m$ ; the net result is about the same. For example, after a 4000 ppm exposure for 8 hr, the reported relationship (16) predicts blood methanol concentrations of 315 mg/L, whereas the model using the standard  $V_{max}$  predicts 174 mg/L. Using the lowest three values of  $V_{max}$  in Table 1 with the corresponding  $K_m$  predicted EOS blood methanol concentrations were 168, 228, and 201 mg/L. These values still represent substantial underpredictions. Hence, variation in  $V_{max}$  with a constant  $K_m$  was considered as the likely cause of the underprediction.

One possible explanation of these apparently anomalous results is that the workers were co-exposed to chemicals that inhibited ADH or otherwise affected methanol elimination. The authors did not note any such co-exposures. Another possible cause of variations in  $V_{max}$  would be that the Japanese workers tested were genetically different from Caucasian subjects used in other studies. Variants of ADH and aldehyde dehydrogenase (ALDH), the principal metabolic enzymes responsible for the metabolism of methanol in primates, are well known in various populations, including the Japanese (27,34). If this were the case for susceptible workers exposed to methanol vapors, the implications for risk assessment are obvious. A worker exposed for 8 hr at the current TLV (200 ppm) with a  $V_{max}$  of 11.5 mg/L/hr (10% of normal  $V_{max}$ ) would experience a BOS concentration the following day of 10.8 mg/L (10 times normal background), which should be easily detected in blood or urine samples.

Another explanation for the anomalous high blood concentrations would be the co-administration of ethanol. All the other data sets described in this paper were gathered from controlled groups, animals, or volunteers. Ethanol is a significant

inhibitor of methanol elimination in primates, due to the higher affinity of ethanol for the ADH enzyme. Equimolar concentrations of ethanol and methanol yield a  $V_{\max}$  for methanol elimination of about 10% of the  $V_{\max}$  in the absence of ethanol; even a 0.25 molar ratio (ethanol to methanol) reduced  $V_{\max}$  to 30% of normal (20). Indeed, this inhibition has been used for treatment of acute methanol toxicity, since ethanol slows the conversion of methanol to formate (1). Furthermore, this inhibition is significant at relatively low doses of ethanol. The current recommended blood concentration of ethanol to treat methanol toxicity is 1000 mg/L (27) or 500 mg/L (1), which approximates intoxicating concentrations following alcohol consumption (800 mg/L is the definition of legal impairment for motor vehicle operators in North Carolina). Methanol elimination may be significantly inhibited in an intoxicated worker without affecting job performance. While the inhibition of methanol elimination by ethanol at relatively low doses is clear, ethanol also is eliminated rather rapidly from the body ( $K_{el}$  for low doses of ethanol is 10 times higher than  $K_{el}$  for methanol). The ethanol co-administration model was developed to examine the relationship between ethanol and methanol regarding inhalation exposure, both as a possible explanation of the high-dose data and for general application in risk assessment. The ethanol co-administration model was based on elimination of ethanol at a constant  $V_{\max}$  and methanol at a variable  $V_{\max}$  as described above.  $\dot{V}$  and  $\Phi$  were 10.5 L/min and 0.75, respectively. Figure 5A presents a model of blood methanol and ethanol compared to methanol alone. The model assumes doses of 30 mL pure ethanol at 0, 4, 8, 12, and 16 hr, and a methanol exposure of 1000 ppm between 0 and 8 hr, for two cases. One case assumes initial BOS blood methanol is unexposed background; the second case adjusts the BOS methanol for each of the preceding 4 days to allow for methanol cumulation; the Monday and Friday 8-hr blood methanol concentrations are 75% to 100% higher than the model predictions without ethanol co-administration. Figure 5B and C demonstrate the same ethanol regimen, with a 200 and 5000 ppm methanol exposure. The 200 ppm exposure shown in Figure 5B indicates that the 8-hr blood methanol concentrations predicted are approximately two-fold greater in the ethanol co-administration predictions, but are still low (less than 20 mg/L maximum) and return to approximately background each day. The 5000 ppm exposure with ethanol co-administration predictions shown in Figure 5C are

42% and 62% higher than without the co-administration. The 8-hr 5000 ppm exposure with ethanol co-administration estimation (321–365 mg/L) approaches the 394 mg/L predicted for that exposure (16).

The model developed (without ethanol co-administration, genetic deficiencies in ADH/ALDH, or other factors that reduce methanol metabolism) does not consider directly the time course of formate (the major metabolite of methanol), and it remains unclear whether it is the parent compound or the metabolite, or some combination of the two, which is responsible for the observed teratogenicity of methanol in laboratory rodents. In any case, the kinetics of the metabolite follow directly from the model, once the transfer of parent from the air into the blood is known. The present model provides that capability, and also illustrates some interesting concepts regarding inhalation toxicology. Consider Table 3, using the proposed benchmark dose of 3078 ppm in the mouse for a 5% added risk of either exencephaly, cleft palate, or fetal resorption (4). Applying a safety factor of 10 to the inhaled concentration for extrapolation between the mouse and the human indicates that a maximum allowable exposure concentration of 308 ppm would be required to protect humans from methanol teratogenicity. If one were to base the factor of safety on the blood concentration, Table 3 indicates that, for a 3078 ppm methanol vapor exposure, mice experience 12- to 18-fold higher blood methanol concentrations than humans. Thus, humans would already have a factor of safety of 13 to 18 at the same exposure concentration. However, considering the proposed benchmark dose for a 5% added risk of cervical rib defects in mice of 305 ppm, and assuming the same 10-fold safety factor, a maximal allowable exposure concentration of about 30 ppm would be required. This would be about the same as if the extrapolation were based on blood concentrations. It should also be noted that, after an 8-hr exposure at the current TLV (200 ppm), the blood methanol concentrations in mice, rats, and humans are approximately the same. The model process and data presented here demonstrate the need to base risk assessment extrapolations between species on actual blood concentrations of the xenobiotic due to the inhalation rather than on environmental vapor concentrations.

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